Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3828–3832, May 1989
Medical Sciences

Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells

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Contributed by Edward A. Boyse, February 9, 1989

ABSTRACT **The purpose of this study was to evaluate human umbilical cord blood as an alternative to bone marrow in the provision of transplantable stem/progenitor cells for hematopoietic reconstitution. Although no direct quantitative assay for human hematopoietic repopulating cells is at present available, the granulocyte–macrophage progenitor cell (CFU-GM) assay has been used with success as a valid indicator of engrafting capability. We examined >100 collections of human umbilical cord blood for their content of nucleated cells and granulocyte–macrophage, erythroid (BFU-E), and multipotential (CFU-GEMM) progenitor cells, in many cases both before and after cryopreservation. First it was determined that granulocyte–macrophage, erythroid, and multipotential progenitor cells remained functionally viable in cord blood untreated except for addition of anticoagulant for at least 3 days at 4°C or 25°C (room temperature), though not at 37°C, implying that these cells could be satisfactorily studied and used or cryopreserved for therapy after transport of cord blood by overnight air freight carriage from a remote obstetrical service. Granulocyte–macrophage progenitor cells from cord blood so received responded normally to stimulation by purified recombinant preparations of granulocyte–macrophage, granulocyte, and macrophage colony-stimulating factors and interleukin 3. The salient finding, based on analysis of 101 cord blood collections, is that the numbers of progenitor cells present in the low-density (<1.077 gm/ml) fraction after Ficoll/Hypaque separation typically fell within the range that has been reported for successful engraftment by bone marrow cells. Another observation of practical importance is that procedures to remove erythrocytes or granulocytes prior to freezing, and washing of thawed cells before plating, entailed large losses of progenitor cells, the yield of unwashed progenitor cells from unfractionated cord blood being many times greater. The provisional inference is that human umbilical cord blood from a single individual is typically a sufficient source of cells for autologous (syngeneic) and for major histocompatibility complex-matched allogeneic hematopoietic reconstitution.**

Circulating blood cells are derived and replaced by a catenated system originating from hematopoietic stem and progenitor cells, recognized mostly by functional tests rather than by morphology (1–4). The primary site of production of stem/progenitor cells in human adults is the bone marrow (1, 2). Hence autologous or major histocompatibility complex-matched bone marrow transplantation is the usual therapeutic vehicle for hematopoietic reconstitution. Although adult blood has had some use as an alternative source (5), in practice the content of stem/progenitor cells is so low (1, 2) that multiple leukopheresis is necessary and has been reported primarily in patients undergoing intense temporary rebound hematopoiesis resulting from recent chemotherapy (5–17).

In human ontogeny, hematopoietic stem/progenitor cells occur first in the yolk sac, later in fetal liver, and then in fetal bone marrow (1, 17), and transplantation of fetal liver cells has been used in a limited setting to correct hematopoietic deficiencies (18, 19). Stem/progenitor cells occur in fetal blood (1, 17), and human umbilical cord blood contains stem cells (20, 21), so called because in colony assay *in vitro* they exhibit replating efficiency indicative of self-renewal, as well as multipotential (CFU-GEMM), erythroid (BFU-E), and granulocyte–macrophage (CFU-GM) progenitor cells (20–26). The frequency of cord-blood progenitors (no. of colonies formed/no. of cells plated) equals or exceeds that of marrow and greatly surpasses that of adult blood. Progenitor cells from human umbilical cord blood can be maintained for many weeks in long-term liquid culture systems, suggesting their production from more primitive cells (22, 27).

The use of human umbilical cord blood for therapeutic reconstitution was proposed by one of us (E.A.B., unpublished work), who subsequently observed successful hematopoietic reconstitution of lethally irradiated inbred mice with syngeneic neonatal blood. The prospect of storing cord blood cells (normally discarded) for potential future medical use by the donor has many advantages, notably in disposing of the need for bone marrow donors, in providing a disease-free source of hematopoietic cells, and in allowing reconstitution of patients who lack a clinically approved donor, freedom from graft-versus-host disease, and freedom from the increased mortality that may accompany nonautologous bone marrow transplantation in circumstances where the degree of accidental hematopoietic injury is uncertain (see refs. 28–30).

The main questions addressed in the following study concern comparative determinations of the reconstituting cellular contents of cord blood and bone marrow, by measure of hematopoietic progenitor cells (31–34) and the collection, transport, and optimal cryopreservation of cord blood.

MATERIALS AND METHODS

Cells. Umbilical cord blood cells were obtained mainly from Bellevue Hospital Center (New York), New York University Hospital (New York), Booth Memorial Center (Flushing, NY), and the Indiana University Medical Center (Indianapolis). The cells were obtained from umbilical cord and placental tissues scheduled for discard, after delivery of

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Abbreviations: CSF, colony-stimulating factor; G, granulocyte; M, macrophage; Epo, erythropoietin; CFU-GM, granulocyte–macrophage colony-forming unit(s); BFU-E, erythroid burst-forming unit(s); CFU-GEMM, granulocyte/erythrocyte/macrophage/megakaryocyte colony-forming unit(s); CM, conditioned medium.

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the infant and after prior needs, if any, for samples for clinical study had been satisfied. The source of each collection was not identified by name or other designation, and *in vitro* hematologic studies were made only as described herein. The Institutional Board of Research Associates of the New York University Medical Center and the Institutional Review Board of Indiana University ruled the collection of blood in these circumstances to be exempt from the consent process.

Immediately upon delivery of the infant, the umbilical cord, in most cases, was doubly clamped 5–7 cm from the umbilicus and transected between the clamps. The infant was removed from the field. Blood was collected from the maternal (placental) end of the transected cord while the placenta remained *in situ* (to take advantage of the enhanced blood flow generated by uterine contraction). In some cases, blood was obtained from the removed placenta by needle aspiration of exposed, engorged vessels on the fetal surface. In all samples collected outside of Indiana, collection was made into a sterile (β-irradiation; Medical Sterilization, Syosset, NY) wide-mouth glass bottle (no. 25625-200; Corning) containing acid-citrate/dextrose (CPD, 20 ml; Sigma) with penicillin (0.03 mg/ml) and streptomycin (0.05 mg/ml) (Sigma) and sent at ambient temperature by overnight express service to the Indiana University School of Medicine for study. In all except 2 of >100 samples, blood samples were received within 24 hr after harvest from the donor. Blood collections in Indiana were made in CPD, ACD (Sigma), or heparin. Cells were left unseparated, were sedimented by gravity or in methylcellulose, or were separated into a low-density fraction (<1.077 gm/ml and, in a few cases, <1.070 gm/ml) by using Ficoll/Hypaque (Pharmacia).

Analysis of Hematopoietic Progenitor Cells *in Vitro*. *Growth factors.* Recombinant preparations of interleukin 3 (specific activity, 10^9 units/mg, Immunex, Seattle, WA), granulocyte (G)-macrophage (M) colony-stimulating factor (CSF) (10^8 units/mg; Immunex), G-CSF (10^8 units/mg; Cetus), M-CSF (5×10^7 units/mg; Cetus) (35), erythropoietin [Epo; Toyoba (New York) or Amgen], and medium conditioned by the human urinary bladder carcinoma cell line 5637 (CM 5637) (36, 37) were used as factors to support the clonal growth of hematopoietic progenitor cells.

CFU-GM. The assay for CFU-GM was performed as described (35, 36) in 0.3% agar culture medium (Difco) that included McCoy's 5A medium supplemented with essential and nonessential amino acids, glutamine, serine, asparagine, vitamins, and sodium pyruvate (GIBCO) with 10% (vol/vol) prescreened heat-inactivated (56°C for 0.5 hr) fetal bovine serum (HyClone) in the absence or presence of CM 5637, interleukin 3, GM-CSF, G-CSF, or M-CSF. Colonies (>40 cells per aggregate) and clusters (3–40 cells per aggregate) were scored after 7 days and 14 days of incubation, as these two scoring days recognize different CFU-GM (38, 39). Large colonies formed (>1000 cells) but results are expressed as colonies plus clusters as this more accurately reflects the total CFU-GM compartment (38).

BFU-E, CFU-GEMM, and CFU-GM. The assay for BFU-E, CFU-GEMM, and CFU-GM was performed in a 1-ml mixture of Iscove's modified Dulbecco's medium (GIBCO), 0.8% methyl cellulose, 30% (vol/vol) prescreened non-heat-inactivated fetal bovine serum (HyClone), 50 μM 2-mercaptoethanol, 0.5–1.0 unit of Epo, with or without 0.1 mM hemin (Eastman Kodak) or 10% (vol/vol) CM 5637 (37). BFU-E, CFU-GEMM, and CFU-GM colonies were scored after 14 days of incubation. BFU-E-1, CFU-GEMM, and CFU-GM assays were scored from the same plates, which include Epo, hemin, and CM 5637. BFU-E-2 were cultured as BFU-E-1 but without CM 5637. BFU-E-2 colonies contained at least 50 cells or were composed of at least three subcolonies containing a minimum of 10 cells each but were usually much larger. Colonies derived from BFU-E-1 were much larger than those derived from BFU-E-2. BFU-E-1 may represent a more immature progenitor than BFU-E-2, although this has not been proven. Overlap in BFU-E-1 and BFU-E-2 colonies between the two assay conditions are likely.

Plating conditions. Cells were plated at concentrations of 0.25, 0.5, and 1.0×10^5 cells per ml for each assay and incubated in a humidified environment with 5% CO_2 at lowered (5%) oxygen tension. Low oxygen tension was maintained using an oxyreducer (Reming Bioinstruments, Redfield, NY) because lowered oxygen tension increases the incidence of detectable progenitor cells (22, 40).

Cryopreservation. Cells were cryopreserved under sterile conditions in two ways. In the initial studies, cells were suspended at a concentration of 4×10^6 cells per ml using a mixture of cold (4°C) 50% (vol/vol) autologous plasma/culture medium and placed on ice. A 1-ml portion of the above-mentioned cell suspension was carefully layered on 1 ml of chilled sterile cryoprotective medium [20% (vol/vol) dimethyl sulfoxide/culture medium] in a cryotube (Nunc). Approximately 10 min prior to freezing, the 1:1 mixture was slowly inverted to promote mixing, then placed on ice to allow equilibrium between the cells and the cryoprotective medium. Vials were placed in a rack in a methanol bath at 4°C just deep enough to cover the cell suspension. This was then placed in the bottom of a freezer at −80°C until cells reached the frozen state. Within 24 hr, the vials were quickly transferred to a container holding liquid nitrogen and placed into the liquid phase. The cell suspension was thawed by gently agitating the vial in a 37°C water bath until a small amount of ice was present. A chilled mixture of 50% (vol/vol) autologous serum/culture medium was asceptically added dropwise with slight mixing between each drop until the suspension volume was doubled. This suspension was transferred to a larger tube with the dropwise addition of the 50% (vol/vol) autologous serum/culture mixture continued until a volume of 6–7 ml was reached. Diluent was then added dropwise, with mixing at every 0.5-ml increment until the volume reached 9–10 ml. Cells were pelleted by centrifugation at 4°C $200 \times g$ for 10 min, the supernatant was aspirated off, and 1 ml of chilled 20% (vol/vol) autologous serum/culture mixture was added dropwise with gentle mixing of the solution. An additional 4 ml of chilled serum/culture medium was added slowly. Cells were washed a second time.

In later studies, larger volumes of cells were placed without separation, concentration, or washing into freezing bags and cooled to −80°C in a liquid nitrogen programmed freezer (Cryomed, Mt. Clemens, MI) coded at an average rate of −1°C per min, as described elsewhere (41), prior to placing the bags for storage in the liquid phase of liquid nitrogen. The defrosting procedure was done without serum-containing medium as the cells were originally frozen in their own plasma.

RESULTS

Survival of Hematopoietic Progenitor Cells. Before evaluating large numbers of umbilical cord blood collections for their content of hematopoietic progenitor cells, experiments were set up to determine whether these cells could survive in cord blood with anticoagulant for up to 3 days without added exogenous growth factors. Cord blood was collected and separated into 10 tubes (2 ml each). One tube was used at time 0 for separation of cells into a low-density fraction (<1.077 gm/ml) and for plating in semisolid medium for assessing progenitor cell numbers. Three tubes each were left at 4°C, ≈25°C (room temperature), and 37°C (in an incubator). On days 1 (t = 24 hr), 2 (t = 48 hr), and 3 (t = 72 hr), one tube from each temperature was used for separation of low-density cells and plating. The data from one or two similar

experiments with reproducible results are shown in Table 1. Overall, the various progenitor cells survived well after 1 day at each of the three temperatures with little or no loss, and some progenitor cell compartments increased in numbers. Progenitor cells survived well also after 2 and 3 days at 4°C and room temperature, but appreciably decreased numbers were seen after 2 days, and no progenitors were apparent after 3 days at 37°C. The survival of hematopoietic progenitor cells may reflect the endogenous production of interleukin 1 by accessory cells (42). Results were similar whether the cells were collected in CPD, ACD, or heparin. These results demonstrated the feasibility of assessing cord blood samples received by overnight express mail service and implied that samples collected even 2 or 3 days previously would contain viable progenitor cells.

Responsiveness of Umbilical Cord Blood to Stimulation by Purified Preparations of Recombinant CSF. To evaluate the types of CFU-GM present in cord blood and whether cord blood contained CFU-GM responsive to the four types of CSF, low-density cord blood cells were plated in the presence of various concentrations of interleukin 3, GM-CSF, G-CSF, and M-CSF. In two experiments, day-14 CFU-GM responded in a dose-dependent manner to stimulation by each of the CSF preparations (Table 2). The number of colonies stimulated by each of the CSF was less than that stimulated by CM 5637. CM 5637 contains a number of growth factors including GM-CSF and G-CSF (37) and the higher number of colonies detected with CM 5637 probably reflects additive or synergistic actions between the growth factors (43).

Numbers of Hematopoietic Progenitor Cells. One hundred and one samples received by overnight express mail service were assessed for a number of parameters, including volume of blood collected, numbers of unfractionated nucleated cells, numbers of low-density nucleated cells, and absolute numbers of hematopoietic progenitor cells in the low-density fraction (Table 3). Although variability was noted between cord blood samples, it was clear that cord blood contained substantial numbers of hematopoietic progenitor cells. In fact, the average number of CFU-GM collected per sample was within the lower range of numbers of CFU-GM in donor marrow that have been associated with successful autologous engraftment (32, 34).

It became apparent, however, that the percent of cells recovered in the low-density fraction, 15.1 ± 0.9% (n = 101 samples), was much lower than was attained with adult bone marrow and circulating adult blood. This low recovery of low-density cord blood cells did not reflect the number of cells loaded on the Ficoll/Hypaque gradient, and removing the red blood cells prior to the density cut did not improve the recovery. Most or all of hematopoietic progenitor cells found in adult bone marrow or blood are isolated in the low-density fraction of cells (refs. 1 and 2; and H.E.B., unpublished observations). Experiments were set up to determine whether the density-cut procedure was also causing loss of hematopoietic progenitor cells. Progenitor cells recovered from unfractionated cord blood (set up without removal of red blood cells) were compared with numbers of progenitors isolated in the low- (<1.077 gm/ml) and high- (>1.077 gm/ml) density fractions. As few as 6–40% of the total number of day-7 and day-14 CFU-GM, BFU-E-2, BFU-E-1, and CFU-GEMM cells were recovered in the low-density fraction. The sum of progenitors in both the low- and heavy-density fractions did not equal that of the unfractionated group and was sometimes as low as 30–40% of the unfractionated blood cell number. Collecting cells with a density of <1.070 gm/ml did not improve the recovery of progenitor cells. Attempts to remove erythrocytes from unfractionated cord blood by lysis of the nonnucleated cells with ammonium chloride or by sedimenting erythrocytes by gravity, methylcellulose, or centrifugation prior to culturing them for assessment of progenitor cell numbers resulted in losses of 50–90% of

Table 2. Responsiveness of CFU-GM in human umbilical cord blood to stimulation by recombinant human CSF

Source	Amount, units	Day-14 colonies, no. per 10^5 low-density cells per ml	
		Exp. 1	Exp. 2
None	—	0	0.5 ± 0.5
CM 5637	—	49 ± 8	31 ± 1
IL-3	100	29 ± 4	16 ± 1
	50	19 ± 2	8 ± 1
	25	17 ± 1	5 ± 1
GM-CSF	200	29 ± 2	16 ± 1
	100	30 ± 1	16 ± 1
	50	28 ± 3	11 ± 2
	25	19 ± 1	5 ± 1
G-CSF	200	37 ± 2	9 ± 1
	100	32 ± 1	7 ± 2
	50	31 ± 1	5 ± 1
	25	21 ± 1	4 ± 1
M-CSF	1000	31 ± 3	10 ± 1
	500	17 ± 1	9 ± 1
	250	14 ± 1	6 ± 2
	125	9 ± 1	4 ± 1

None is the background value. CM-5637 was added at 10% (vol/vol).

Table 1. Influence of time and temperature on survival of nucleated cells and hematopoietic progenitor cells in human umbilical cord blood

Time, days	Temp.	Low-density nucleated cells, no. × 10^{-6}	Hematopoietic progenitor cells, total number					
			Agar culture		Methylcellulose culture			
			Day-7 CFU-GM	Day-14 CFU-GM	Day-14 CFU-GM	BFU-E-2	BFU-E-1	CFU-GEMM
0		9.3	5952	8556	10,416	4,092	4464	744
1	4°C	5.7	3420	7296	15,048	5,244	7980	684
1	25°C	7.9	4108	8848	10,428	4,740	6004	632
1	37°C	13.5	4320	7290	13,500	5,670	5940	810
2	4°C	6.9	3588	6900	13,248	7,452	4140	552
2	25°C	8.4	3912	7392	19,488	10,752	8400	1344
2	37°C	0.9	144	324	1,152	360	360	0
3	4°C	7.6	3648	6536	18,544	3,040	7296	152
3	25°C	8.6	2752	5848	19,608	4,816	9976	688
3	37°C	0	0	0	0	0	0	0

Cord blood was collected and assessed for survival. Day-7 and -14, CFU-GM in agar cultures were stimulated with 10% (vol/vol) CM 5637. BFU-E-2 cultures were stimulated with 1 unit of Epo plus 0.1 mM hemin and BFU-E-1, CFU-GEMM, and day-14 CFU-GM in methylcellulose cultures were stimulated with 1 unit of Epo, 0.1 mM hemin, plus 10% (vol/vol) CM 5637. Temp., temperature.

Table 3. Nucleated cellularity of unfractionated human umbilical cord blood and numbers of nucleated cells and hematopoietic progenitor cells present in the low-density fraction of cord blood

Parameter evaluated	Value	No. of samples
Blood collected, ml	56.3 ± 2.4	101
Unfractionated nucleated cells, no. × 10^{-6}	784 ± 59	101
Low-density nucleated cells, no. × 10^{-6}	126 ± 14	101
Hematopoietic progenitor cells, no. × 10^{-5}		
Agar culture		
Day-7 CFU-GM	0.42 ± 0.06	101
Day-14 CFU-GM	1.10 ± 0.16	101
Methylcellulose culture		
Day-14 CFU-GM	2.34 ± 0.39	85
BFU-E-2	0.94 ± 0.15	91
BFU-E-1	0.86 ± 0.13	91
CFU-GEMM	0.51 ± 0.08	91

Progenitor cell assays were set up as described in text and in the legend to Table 1. Values are mean ± 1 SEM.

progenitor cells. These data suggested that actual numbers of hematopoietic progenitor cells in human umbilical cord blood had been largely underestimated and that the most accurate way to make this estimate was to use unfractionated cord blood.

With this information in mind, attempts were made to optimize the amount of cord blood collected, and also to recover blood from the placenta after the baby had been delivered. Results from three cord blood collections made in this way, which assessed progenitor cell numbers in unseparated blood, are shown in Table 4. These studies illustrate much greater yields of nucleated and hematopoietic progenitor cells. The yields of nucleated cells recovered are well within the range of cell numbers usually used for autologous and allogeneic bone marrow transplantation, and the numbers of CFU-GM are in the upper range of the numbers of CFU-GM in donor marrows that have been associated with successful engraftment in autologous bone marrow transplantation and are in the range used for allogeneic transplantation (31–34).

Cryopreservation of Umbilical Cord Blood Cells. Twelve samples of low-density (<1.077 gm/ml) cord blood cells were frozen in small tubes in 2-ml aliquots of 8 × 10^6 cells using the methanol freezing technique. Cells were thawed after 1–10 months and washed twice, and viable nucleated cells were counted and plated for hematopoietic progenitor cell quantitation. The recoveries for thawed samples (mean ± SEM) of nucleated cells, day-7 and -14 CFU-GM (agar cultures), day-14 CFU-GM, BFU-E-2, BFU-E-1, and CFU-GEMM (methylcellulose cultures) were, respectively, 35.0 ± 4.5%, 66.6 ± 10.1%, 63.1 ± 7.3%, 45.8 ± 9.6%, 44.9 ± 4.9%, 41.3 ± 6.2%, and 30.4 ± 2.6%. Addition of 40–100 units of DNase to the samples during thawing to reduce cell clumping, slightly [but not significantly ($p > 0.05$)] enhanced recovery by 12–20%. Unseparated cells that were frozen, thawed, and washed had the same percent recovery as the frozen low-density cells (three experiments, $p > 0.05$). Recovery was similar whether cells were thawed 1 or 10 months after freezing.

Table 4. Nucleated cellularity and numbers of hematopoietic progenitor cells present in unfractionated human umbilical cord blood

Parameter evaluated	Value
Blood collected, ml	197 ± 42
Nucleated cells, no. × 10^{-6}	2400 ± 1200
Hematopoietic progenitor cells, no. × 10^{-5}	
Agar culture	
Day 14 CFU-GM	19.0 ± 15.2
Methylcellulose culture	
Day 14 CFU-GM	38.0 ± 35.3
BFU-E-2	17.9 ± 10.4
BFU-E-1	19.1 ± 9.6
CFU-GEMM	8.1 ± 2.6

Values are from three donor samples and expressed as mean ± 1 SEM. Progenitor cell assays were set up as described in text and in the legend to Table 1.

In further experiments, 80–100% of nucleated cells were recovered in a viable state immediately after thawing, but any washing of the cells to remove dimethyl sulfoxide decreased the recovery rate. To freeze large numbers of unseparated cells, the cryopreservation protocol was modified to use freezing bags holding up to 100 ml of the cell preservation medium mixture and to use a timed freezing apparatus as described elsewhere (41). Small aliquots of cells were thawed, counted, and plated without washing. The starting concentration of unseparated cells frozen (8–20 × 10^6 cells per ml) allowed the plating of cells at a concentration (2.5–5 × 10^4 cells per ml) such that the actual amount of dimethyl sulfoxide added to the 1-ml culture dishes were diluted below the concentration that would interfere with the growth of the progenitor cells. In three experiments, the recoveries of day-14 CFU-GM, BFU-E-1, and CFU-GEMM, as assessed in methylcellulose cultures, were, respectively, 100%, 40–60%, and 75–100% from 1 month to 6 months after the initial freezing. These results indicate that large numbers of nucleated and hematopoietic progenitor cells from human umbilical cord blood can be cryopreserved and retrieved with good recovery.

DISCUSSION

We see that blood collected from the umbilical cord contains CFU-GM in numbers well within the range of marrow CFU-GM that have been associated with successful autologous and major histocompatibility complex-matched allogeneic bone marrow transplantation (31–34). Although the CFU-GM is not a stem cell, but rather a lineage-restricted progenitor cell, the speed of hematologic recovery and survival of mice transplanted with bone marrow have been correlated directly with the number of CFU-GM transplanted (44), and the CFU-GM content of human bone marrow also correlated with the rate of hematopoietic reconstitution after autologous bone marrow transplantation (45). Cord blood BFU-E and CFU-GEMM were also within the range of these progenitors found in successful human marrow donor grafts (31).

Unfortunately, there is not yet a direct assay for human hematopoietic repopulating cells. Pluripotential hematopoietic stem cells are defined functionally by their ability to self-renew (make more of themselves) and to give rise to blood cells of multilineages (1). Murine colony assays *in vivo* detect what appear to be subsets of myeloid stem cells, the colony-forming units in spleen (CFU-S) (1, 3, 43). Murine CFU-S have self-renewal capacity and give rise to multicell lineages, but it does not appear that the CFU-S is a marrow-repopulating cell (1, 3, 43). The closest human equivalent to the murine CFU-S *in vitro* is the stem (S) cell (46, 47). Human umbilical cord blood contains *in vitro* stem cells (20, 21), which were identified in human cord blood before they were detected in human bone marrow (48–50). The frequency of stem cells in both human cord blood and human marrow is essentially the same, but their numbers are very low (20, 21, 48–50). Also, human cord blood stem cells and human bone marrow stem cells do not show the same degree of replating efficiency *in vitro* as does the murine stem cell (20, 21, 46–50). The human stem cell assay is not suited for routine quantitation because

of its low frequency and also because colonies deriving from stem cells are detected retrospectively, after it has been shown that the colony scored as being stem cell-derived contains cells capable upon replating of giving rise to secondary colonies containing cells of multilineages. The human CFU-GEMM, because it has little or no replating efficiency *in vitro*, is considered to be a multilineage progenitor cell rather than a stem cell (1, 3, 43). There is no evidence that numbers of CFU-GEMM present in a donor inoculum are a better predictor of engraftment potential than are the numbers of CFU-GM (51).

Our results suggest that umbilical cord blood from a single donor could serve as a source of autologous or major histocompatibility complex-matched allogeneic transplantable hematopoietic repopulating cells. These cells have been cryopreserved and hematopoietic progenitor cells, CFU-GM, BFU-E, and CFU-GEMM, have been retrieved in a functionally viable form. In this context, it is clear that the cord blood should not be separated to remove any cell types prior to freezing and should not be washed or otherwise manipulated after thawing since all such procedures caused severe losses of hematopoietic progenitor cells. Fortunately, there have already been clinical situations in which unseparated marrow cells have been frozen, thawed, and infused into recipients without washing. Marrow aspirated for bone marrow transplantation, because of the large amounts of cells needed, is usually diluted with blood and, therefore, would contain more erythrocytes than are normally present in marrow. It therefore seems reasonable to assume that cord blood infused into donors immediately after thawing would not present serious problems.

The final question of whether human cord blood cells can successfully reconstitute a human subject is the subject of an international and multiinstitutional collaboration (E. Gluckman, H.E.B., A. D. Querbach, H. S. Friedman, G.W.D., A. Devergie, H. Esperou, D. Thierry, G. Socie, P. Lehn, S.C., D.E., J. Kurtzberg, J.B., and E.A.B., unpublished research).

We thank Linda Cheung for typing the manuscript. These studies were supported by a grant from the Biocyte Corporation (New York, NY) and by Public Health Service Grants CA36464 and CA36740 (to H.E.B.) from the National Cancer Institute.

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